PAPER CHROMATOGRAPHY AND CHEMICAL STRUCTURE

III. THE CORRELATION OF COMPLEX AND SIMPLE MOLECULES. THE CALCULATION OF R_M VALUES FOR TOCOPHEROLS, VITAMINS K, UBIQUINONES AND UBICHROMENOLS FROM R_M(PHENOL). EFFECTS OF UNSATURATION AND CHAIN BRANCHING

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INTRODUCTION

It was shown in the previous communication[1] that MARTIN's equation can be extended to accommodate constitutive effects in molecules. If sufficient R_M data are available from the study of relevant compounds, many constitutive effects can be analysed in terms of ΔR_M parameters, which can then be used to calculate R_M values. Thus, previously[1], the R_M values of a large number of low molecular weight phenols, alkoxyphenols, coumaranols and chromanols were correlated with their structure and with the R_M value of a standard compound, which, in all these investigations, we have taken as phenol. The aim of the investigations described here was to correlate the chromatographic behaviour of more complex molecules, such as the tocopherols, ubiquinones and ubichromenols, with their chemical structure, and to relate them also to the same standard reference compound, phenol. Several new problems must be solved before this can be done: they largely, but not entirely, spring from the experimental limitation that, since these substances are of fairly high molecular weight, they cannot be chromatographed in any system suitable for the low molecular weight phenols. The most complex phenol it was possible to chromatograph in System 1 contained fourteen carbon atoms[1], whereas α-tocopherol contains twenty-nine and ubiquinone 50 fifty-nine carbon atoms. In order to correlate such compounds with phenol, therefore, the following general procedure must be followed. First, every additive group and every constitutive effect in the required complex molecule must be analysed chromatographically and its ΔR_M parameter determined in a suitable system. Secondly, the complex molecule must be chromatographically correlated with the simple molecule by studying compounds intermediate in structure and molecular weight in a progressive series of chromatographic bridging systems. By these means, the ΔR_M parameters can be calculated for the series of systems and from them R_M values for progressively more complex molecules. These values can then be incorporated in the calculation of the R_M value of the required molecule.

Before it was possible to proceed with confidence, it appeared necessary to answer several pertinent questions. Although it was shown previously[1] that MARTIN's equation is obeyed with respect to several atomic and group ΔR_M parameters, when

a series of related compounds are chromatographed in one system, it was not certain that this would be so for other systems. LEDERER[2] has summarised considerable evidence to show that $\Delta R_M(CH_2)$ is not only constant when a number of different chemical series are run in the same system but that MARTIN's equation continues to be obeyed when the same series is run in several different systems. However, as we have already discussed[1], it has on occasion been suggested that the value of ΔR_M for a group may vary according to the remainder of the molecule—that is, even in the absence of constitutive interaction, ΔR_M in a large molecule might be different from ΔR_M in a small molecule. In view, therefore, of the large differences between the molecular weights of the compounds used in this investigation and the diverse nature of the systems employed, it was essential to investigate the constancy of ΔR_M for groups other than CH_2 and also for constitutive effects.

To this end, a number of new compounds were synthesised and chromatographed in several reversed phase partition systems.

PREPARATION OF COMPOUNDS

(a) *Mono-ethers of hydroquinones (alkoxyphenols)*

These were made by the general procedure described in the preceding paper[1], except where stated. A number have been described previously[3]. The following were new compounds: *p-n-tetradecyloxyphenol*, m.p. 84°; *p-n-hexadecyloxyphenol*, m.p. 88°; *p-n-octadecyloxyphenol*, m.p. 91.5°; *p-(hex-4-enyloxy)-phenol*, characterised as the *p-nitrophenylurethane*, m.p. 150°; *p-(1-methylbutoxy)-phenol*, b.p. 110–120°/0.5 mm; *p-(2-methylbutoxy)-phenol*, 80–100°/0.15 mm; *p-(1-methylpentyloxy)-phenol*, b.p. 120–140/0.2 mm; *p-(1-ethylbutoxy)-phenol*, b.p. 110–130°/0.15 mm; *p-(1-ethylpentyloxy)-phenol*, b.p. 105°/0.1 mm; *p-(1-propylbutoxy)-phenol*, b.p. 120°/0.15 mm; *p-(1-methylheptyloxy)-phenol*, b.p. 110–120°/0.5 mm; *p-citronellyloxyphenol*, b.p. 150°/0.2 mm; *p-dihydrocitronellyloxyphenol*, b.p. 135–145°/0.6 mm; *p-hexahydrofarnesyloxyphenol*, b.p. 162°/0.1 mm, n_D^{20} 1.4965; *p-dihydrophytyloxyphenol*, b.p. 184°/0.1 mm, n_D^{19} 1.4890; *p-(6-cyclohexylhexyloxy)-phenol*, m.p. 56–58°.

p-(Hexa-2,4-dienyloxy)-phenol (*p-sorbyloxyphenol*) and *p-geranyloxyphenol* were oils that could not be purified by distillation since they are allylic ethers and undergo thermal rearrangement. However, the former compound analysed correctly after chromatography, whilst the latter was characterised as the *p-nitrophenylurethane*, m.p. 117–118°.

5-Dihydrophytyl-4-methoxy-2-methylphenol was prepared by hydrogenation of the previously described 4-methoxy-2-methyl-5-phytylphenol[4], and had b.p. 180°/0.05 mm.

(b) *Alkoxyphenyl benzoates*

Most of the benzoates used in this study are known compounds and were prepared by normal methods from the phenols and alkoxyphenols. The following three benzoates were new compounds and analysed correctly: *p-n-butoxyphenyl benzoate*, had m.p. 71.5°; *p-sec.-butoxyphenyl benzoate* had m.p. 53°; *p-tert.-butoxyphenyl benzoate* had m.p. 92.5°.

(c) *Tocopherol ethers*

These were prepared by Williamson synthesis. *Tocol allyl ether* was a pale yellow oil and distilled in a short-path still at 160–170° (bath)/$5 \cdot 10^{-3}$ mm (Pirani); λ_{max} 295 mμ

($E^{1\%}_{1cm} = 81.5$); λ_{min} 255 mμ. *β-Tocopherol allyl ether* was obtained similarly; λ_{max} 292.5 mμ ($E^{1\%}_{1cm} = 72.0$); λ_{min} 260 mμ. *δ-Tocopherol allyl ether* was obtained similarly; λ_{max} 295 mμ ($E^{1\%}_{1cm} = 82.5$); λ_{min} 260 mμ.

(d) Ubiquinones and ubichromenols

Ubiquinones 30 and 50 were the generous gift of Hoffmann-La Roche Laboratories, Basle, Switzerland. Perhydroubiquinone 50 has been described[5]. *Dodecahydroubiquinone 30* was prepared in an analogous fashion by reduction of ubiquinone 30, followed by re-oxidation to the quinone; it was not further characterised. Ubiquinol 30 and dodecahydroubiquinol 30 were obtained by reduction of ubiquinone 30 and perhydroubiquinone 30 respectively with potassium borohydride. Hexahydroubiquinone 20 was prepared by the method of SHUNK *et al.*[6]. Hydrogenation followed by re-oxidation to the quinone gave *octahydroubiquinone 20*, which was not further characterised. Hexahydroubichromenol 20 and hexahydroubichromanol 20 have been previously described[6,7].

PAPER CHROMATOGRAPHIC METHODS

Several new chromatographic systems were used to study the higher molecular weight substances. Increasing strengths of aqueous ethanol were used as the mobile phase. Ethyl oleate—used previously with the simple phenols[1]—was now no longer suitable as stationary phase, being too soluble in these increased concentrations of ethanol. It was replaced by olive oil, whose properties are similar to those of ethyl oleate but which is almost insoluble in all but the highest concentrations of ethanol. Olive oil possessed certain advantages over liquid paraffin or petroleum jelly as stationary phase. Being slightly more polar than the latter, ΔR_M increments for carbon were rather smaller and more substances could be run in any one system; this characteristic is especially desirable in structural studies. For compounds with the highest molecular weights, it was necessary to use liquid paraffin for the stationary phase, this substance remaining satisfactory even when pure ethanol was used as the mobile phase. Paraffin/alcohol systems give the largest ΔR_M increments for groups such as CH_2: they are thus especially useful for the discernment of small molecular weight differences between compounds, but have the corresponding disadvantage that the range of compounds run on one chromatogram must be restricted.

Phenols, esters and ethers were visualized on the paper by the methods described previously[1]. The unsaturated long-chain alcohols were visualized by treatment with sulphuric acid. The ubiquinones, ubichromenols, tocopherols, vitamins K and analogous compounds were observed under ultra-violet light as dark spots. All the polynuclear hydrocarbons appeared as dark spots under ultra-violet light, except anthracene, which was brightly fluorescent.

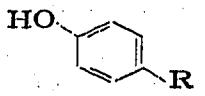
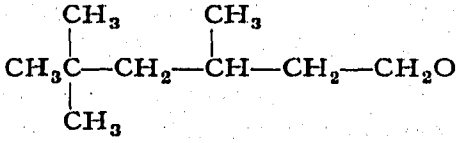
RESULTS WITH SYSTEM 2

System 2 was 70% (v/v) ethanol against olive oil, and Table I records the R_M values for 54 compounds run in this system. They included phenols, hydroquinone monoethers ranging from *n*-butoxyphenol (No. 5) to *n*-octadecyloxyphenol (No. 16), phenyl benzoates, phenyl nitrobenzyl ethers and several important long-chain isoprenoid alcohols. System 2 provided an important bridge between System 1 (25%

TABLE I

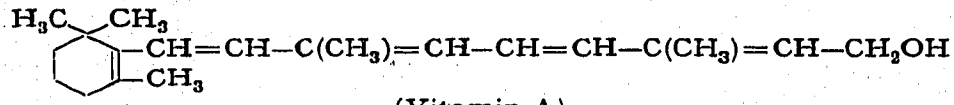
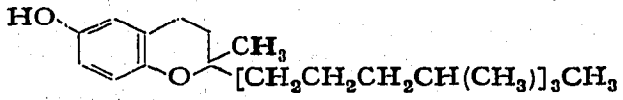
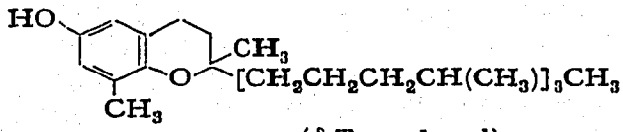
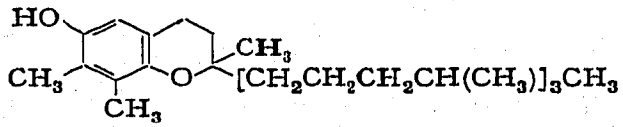
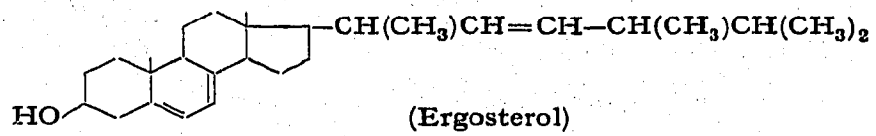
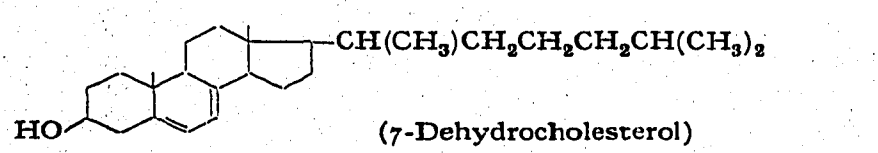
CHROMATOGRAPHY OF PHENOLS, HYDROQUINONE MONO-ETHERS, PHENOL BENZOATES, NITROBENZYL ETHERS AND SOME ISOPRENOID ALCOHOLS IN SYSTEM 2

Stationary phase: Whatman No. 4 paper impregnated with a 5% (v/v) solution of olive oil in light petroleum (40–60°).
Mobile phase: 70% (v/v) ethanol in water.

No.	Compound	R_F	R_M
	I. R in structure shown 		
	(a) Phenols		
1	n-C_3H_7	0.85	—0.740
2	n-C_4H_9	0.80	—0.618
3	n-C_5H_{11}	0.75	—0.483
4	$CH_3CH(CH_3)CH_2CH_2$	0.75	—0.483
	(b) Hydroquinone mono-ethers with straight-chain alkoxy groups		
5	n-C_4H_9O	0.85	—0.736
6	$CH_3CH(CH_3)CH_2CH_2O$	0.80	—0.600
7	n-$C_6H_{13}O$	0.75	—0.476
8	n-$C_7H_{15}O$	0.69	—0.347
9	n-$C_8H_{17}O$	0.62	—0.215
10	n-$C_{11}H_{23}O$	0.40	+0.174
11	n-$C_{12}H_{25}O$	0.335	+0.296
12	n-$C_{14}H_{29}O$	0.22	+0.554
13	$CH_2=CHCH_2CH_2CH_2O$	0.83	—0.688
14	$CH_3CH=CHCH_2CH_2CH_2O$	0.78	—0.550
15	n-$C_{16}H_{33}O$	0.13	+0.811
16	n-$C_{18}H_{35}O$	0.08	+1.066
	(c) Hydroquinone mono-ethers with ring-containing alkoxy groups		
17	Phenyloxy	0.81	—0.638
18	Benzyloxy	0.83	—0.678
19	Cyclopentyloxy	0.86	—0.799
20	Cyclohexyloxy	0.83	—0.678
21	6-Cyclohexylhexyloxy	0.37	+0.228
	(d) Hydroquinone mono-ethers with branched alkoxy groups		
22		0.625	—0.225
23	$H[CH_2C(CH_3)=CHCH_2]_2O$ (Geranyloxy)	0.64	—0.255
24	$(CH_3)_2C=CHCH_2CH_2CH(CH_3)CH_2CH_2O$ (Citronellyloxy)	0.60	—0.180
25	$H[CH_2CH(CH_3)CH_2CH_2]_2O$ (Dihydrocitronellyloxy)	0.56	—0.100
26	$H[CH_2CH(CH_3)CH_2CH_2]_3O$ (Hexahydrofarnesyloxy)	0.27	+0.431
27	$H[CH_2CH(CH_3)CH_2CH_2]_4O$ (Dihydrophytyloxy)	0.10	+0.947
	II. Benzoates of phenols and hydroquinone mono-ethers		
28	Phenyl benzoate	0.49	+0.009
29	p-Tolyl benzoate	0.42	+0.140
30	3,4-Dimethylphenyl benzoate	0.35	+0.267
31	3,5-Dimethylphenyl benzoate	0.35	+0.267
32	p-Ethylphenyl benzoate	0.35	+0.274

(continued on p. 162)

TABLE I (*continued*)

No.	Compound	R_F	R_M
33	*p-n*-Propylphenyl benzoate	0.28	+0.408
34	*p-tert.*-Butylphenyl benzoate	0.25	+0.474
35	*p-n*-Butylphenyl benzoate	0.23	+0.533
36	*p-n*-Butoxyphenyl benzoate	0.29	+0.400
37	*p-sec.*-Butoxyphenyl benzoate	0.33	+0.316
38	*p-tert.*-Butoxyphenyl benzoate	0.37	+0.204
39	5,6,7,8-Tetrahydro-2-naphthyl benzoate	0.27	+0.428
40	4-Diphenyl benzoate	0.21	+0.574
41	2-Naphthyl benzoate	0.295	+0.380
	III. Phenyl nitrobenzyl ethers		
42	Phenyl *p*-nitrobenzyl ether	0.51	−0.020
43	*p*-Tolyl *p*-nitrobenzyl ether	0.44	+0.107
	IV. Isoprenoid alcohols and tocols		
44	$(CH_3)_2C=CHCH_2CH_2C(CH_3)=CHCH_2OH$ (Geraniol)	0.90	−0.918
45	$(CH_3)_2C=CHCH_2CH_2CH(CH_3)CH_2CH_2OH$ (Citronellol)	0.87	−0.837
46	$(CH_3)_2C=CHCH_2CH_2C(CH_3)=CHCH_2CH_2C(CH_3)=CHCH_2OH$ (Farnesol)	0.75	−0.468
47	$(CH_3)_2C=CHCH_2CH_2C(CH_3)=CHCH_2CH_2C(CH_3)CH=CH_2$ (Nerolidol)	0.68	−0.328
48	$H[CH_2CH(CH_3)CH_2CH_2]_3CH_2C(CH_3)=CHCH_2OH$ (Phytol)	0.38	−0.218
49	 (Vitamin A)	0.52	−0.040
50	 (Tocol)	0.135	+0.806
51	 (δ-Tocopherol)	0.10	+0.941
52	 (γ-Tocopherol)	0.08	+1.074
53	 (Ergosterol)	0.10	+0.947
54	 (7-Dehydrocholesterol)	0.09	+1.016

ethanol against ethyl oleate)[1] and the systems described later for use with more complex substances. The main aims of the study with System 2 were to investigate the ΔR_M parameters studied previously[1], to discover how they changed with the change in the system, and to confirm that they remain constant irrespective of the nature of the molecular series. Several phenols and alkoxyphenols were selected and converted into their benzoates and (in a limited number of cases) into their *p*-nitrobenzyl ethers. The compounds could all be chromatographed together in System 2 and, by this means, it was possible to compare ΔR_M increments in virtually identical molecular surroundings, but in different series of compounds running to different points of the same chromatogram. In addition, ΔR_M parameters were checked for constancy in series varying more widely in structure.

$\Delta R_M(CH_2)$ and ΔR_M(ring-attached CH_2) parameters

$\Delta R_M(CH_2)$ was calculated independently from comparison of compounds in three series; phenols, *p*-alkoxyphenols and their respective benzoates. It was found to be $+ 0.129 \pm 0.005$. The results in Table I confirm that the additivity rule is strictly obeyed over a range of ten carbon atoms (*p*-butoxyphenol to *p*-tetradecyloxyphenol) and that $\Delta R_M(CH_2)$ is constant irrespective of the remainder of the molecule, providing constitutive interactions are absent. The value of ΔR_M (ring-attached CH_2) can be calculated, in the usual way, by comparing (i) the benzoates and (ii) the nitrobenzyl ethers of phenol and *p*-cresol. It also is $+ 0.129 \pm 0.003$. Thus, in contrast to the findings[1] in System 1, the two parameters are identical. This arises from the fact that in System 2, $\Delta R_M(CH_2)$ is small. If there was the same relative difference between the two parameters as there was in System 1, it would only amount to about 0.030 in System 2, which is just about experimental error. (Note, however, that different ΔR_M parameters do not by any means change in constant proportion when the system is changed. It would not even seem to be a theoretical requirement that they should all change in the same *direction* when the system is changed—in the chromatography of amino acids, for instance, "crossover" of spots in different systems is familiar. Nevertheless, for the systems studied here, which were all fairly similar and usually involved only an alteration in the mobile phase concentration, the order of change of the limited number of ΔR_M parameters investigated was indeed similar.)

$\Delta R_M(H)$ parameters and the effect of unsaturation

It follows from the fact that $\Delta R_M(CH_2)$ and ΔR_M(ring-attached CH_2) are virtually identical in System 2 not only that the atomic $\Delta R_M(H)$ parameter is small in this system, but that the differences between the $\Delta R_M(H)$ values for hydrogen α, β, γ, etc. to the ring are much too small to be determined with any accuracy (compare System 1[1]). Thus, the R_M values of the two xylenol benzoates (Nos. 30 and 31) are virtually the same as that of *p*-ethylphenyl benzoate (No. 32) and the ΔR_M increment between phenyl benzoate and *p*-cresyl benzoate (Nos. 28 and 29) is almost identical with the R_M difference between *p*-propylphenyl benzoate and *p*-butylphenyl benzoate (Nos. 33 and 35). Nevertheless, although the *difference* between the $\Delta R_M(H)$ parameters at different positions α, β, γ, etc. from the ring is too small to determine, that it still exists in System 2 is clearly illustrated by the fact that *p-tert.*-butylphenyl benzoate (No. 34), which contains no α-hydrogens, runs a little faster than *p-n*-butylphenyl benzoate (No. 35), the R_M difference being 0.059. (This difference is unlikely to

be due to chain branching in the former substance (see later), since the R_M values for *p*-*n*-amylphenol (No. 3) and *p*-3-methylbutylphenol (No. 4) are identical.)

In System 2, therefore ΔR_M(H) can, for all practical purposes, be considered to be a constant, irrespective of the position of the hydrogen with respect to the ring. Its value, although small, can be determined with reasonable accuracy. For example, the R_M values of *p*-(pent-4-enyloxy)-phenol (No. 13) and *p*-(3-methylbutyloxy)-phenol (No. 6) differ by + 0.088, and the R_M values of *p*-(hex-4-enyloxy)-phenol (No. 14) and *p*-hexyloxyphenol (No. 7) differ by + 0.074. The introduction of a double bond into compounds 6 and 7 therefore decreases their R_M values by about — 0.080. This corresponds to a value of + 0.040 for ΔR_M(H). The same value for ΔR_M(H) is found if the higher molecular weight compounds of the hydroquinone mono-ether series are compared. Thus *p*-geranyloxyphenol (No. 23), *p*-citronellyloxyphenol (No. 24) and *p*-dihydrocitronellyloxyphenol (No. 25), which differ from each other by two hydrogen atoms, differ in R_M by about 0.080. The formation of an alicyclic ring also corresponds formally to the loss of two hydrogen atoms. Chromatographically, therefore, the presence of an alicyclic ring should correspond to a double bond, provided that the ring is not attached to oxygen, when other effects appear (see later). This was shown to be the case by comparing *p*-(6-cyclohexylhexyloxy)-phenol (No. 21) with *p*-*n*-dodecyloxyphenol (No. 11), whose R_M values differ by + 0.068. The same value for the ΔR_M(H) parameter was found in the series of isoprenoid alcohols; here, the R_M values of geraniol (No. 44) and citronellol (No. 45) differed by + 0.081. There is thus ample evidence from Table I that ΔR_M(H) is a chromatographic constant. Hence, in the absence of any interaction with other structures, ΔR_M(double bond) must be constant.

Because the atomic ΔR_M parameters for both carbon and hydrogen are small in System 2, little loss of accuracy is introduced if group $\Delta R_M(CH_2)$ parameters are used for the calculation of R_M values of compounds run in this system. It follows, for instance, that if the R_M value of *n*-octylphenol is to be calculated from R_M(phenol), the error introduced by ignoring the fact that α- and β-CH_2 groups are chromatographically slightly different from subsequent CH_2 groups in the alkyl chain is negligible: as the molecular weights of the compounds increase, the contributions of α- and β-hydrogens become correspondingly less. In all the calculations below, therefore, the CH_2 group parameter has been employed.

ΔR_M parameters for oxygen in ethers

The results in Table I show that the R_M values for *p*-*n*-butoxy-, *p*-*sec*.-butoxy- and *p*-*tert*.-butoxyphenyl benzoates (Nos. 36, 37 and 38) in System 2 decrease in the same order as was found previously[1] in System 1; indicating, therefore, that ΔR_M(O) varies in the same way, according to the nature of the alkyl group to which it is attached.

The *group* ΔR_M parameters for oxygen can be calculated, exactly as described earlier[1], by directly comparing the R_M values for the primary, secondary and tertiary butoxyphenyl benzoates (Nos. 36, 37 and 38) with the R_M value for the corresponding primary butylphenyl benzoate (No. 35). The respective values are as follows: ΔR_M (O in OCH_2R) — 0.133; ΔR_M(O in $OCHR_2$) — 0.217; and ΔR_M(O in OCR_3) — 0.329. (It should be noted that these *group* ΔR_M parameters are slightly in error, for reasons that have been discussed in the preceding paper[1]. Briefly, ΔR_M increments for oxygen should ideally be calculated by the methods of atomic parameters outlined previously, or else they do not take into account the variation of the R_M(H) incre-

ments for hydrogen α, β and γ to the ring in the alkyl group and the absence of such variation in the alkoxy group. However, since in System 2 the differences are known to be small, it is unlikely that any serious effect on calculations would be introduced by using these group oxygen parameters—as would certainly have been the case in System 1.) All the other effects of substitution vicinal to oxygen are the same in System 2 as they were in System 1. Thus *p*-phenyloxyphenol (No. 17) runs slightly *slower* than *p*-benzyloxyphenol (No. 18), although the latter contains one more CH_2 group, because of the "resonance" effect of the benzyl group. The following calculation shows that *p*-cyclohexyloxyphenol (No. 20) is slightly faster in System 2 than required by theory, as it was in System 1.

Calculation of R_M(cyclohexyloxyphenol)

R_M(cyclohexyloxyphenol) = R_M(*n*-hexyloxyphenol) — 2 × ΔR_M(H) — (a correction for cyclohexyloxyphenol being a secondary ether)

The correction for a secondary ether is obtained by subtracting the R_M value of *p-sec.*-butoxyphenyl benzoate from that of *p-n*-butoxyphenyl benzoate. It is *subtracted* in the above calculation, since a secondary ether runs faster than a primary ether.
Thus,

R_M(cyclohexyloxyphenol) = — 0.476 — (2 × 0.040) — (— 0.400 — 0.316)
= — 0.640
Experimental R_M = — 0.678

Similarly, *p*-cyclopentyloxyphenol (No. 19; experimental R_M = — 0.799) runs slightly faster than required by theory (calculated R_M = — 0.771). The reason for this has been already discussed[1]. It should be noted that in System 2, the differences are only just outside experimental error. Nevertheless, they are of the same order, compared to the value of $\Delta R_M(CH_2)$, as they were in System 1.

The ΔR_M(OH) parameter

Since all the compounds in Table I, except the isoprenoid alcohols and vitamin A (Nos. 44–49), are phenols or their derivatives, the ΔR_M parameter for the phenolic OH group is already included in the R_M value for the ground molecule. (It could be calculated, if required, by comparing a suitable series of phenols and hydroquinones, although we have not done this.) It should be noted, however, that the values of ΔR_M(alcoholic OH)—in the series of isoprenoid alcohols—obviously depends on whether the alcohol is primary or tertiary. This is the same effect as occurs with ΔR_M(O) in the hydroquinone mono-ether series. But, in the alcohol series, the primary alcohol farnesol runs *faster* than its tertiary isomer, nerolidol. This is to be expected, since now it is oxygen–hydrogen polarization that is the determining factor, not carbon–oxygen polarization.

The effect of chain branching on R_M values

Many of the higher molecular weight compounds to be studied in later systems, especially the naturally-occurring tocopherols and ubiquinones, are isoprenoid in structure and their molecules contain branched alkyl chains. In System 1, most of the compounds studied were unbranched: the others were low-molecular weight com-

pounds and, if they did contain a single branch, any effect it might have had was obscured by the pronounced effects of α- and β-hydrogen atoms on the R_M values of the compounds. Thus, although *p*-isopropylphenol ran faster than *p*-*n*-propylphenol, and *p*-*tert.*-butylphenol ran faster than *p*-*n*-butylphenol[1], this was solely due to the fact that each new branching at carbon replaced, in effect, an α-hydrogen by a β-hydrogen. In compounds where this could be discounted (that is, where the branching occurred beyond the γ-carbon atom) branching as such did not appear to affect chromatographic behaviour in these simple compounds. Thus, in System 1, *p*-amylphenol was inseparable from *p*-3-methylbutylphenol, and *p*-cyclopentylphenol was inseparable from its isomer, *p*-3-methylbut-2-enylphenol[1].

It was clearly necessary, however, to study the effect of multiple chain branching in more detail. This was done in System 2, and it soon became apparent that the behaviour of compounds containing long-branched chains was anomalous. For example, the R_M value (— 0.100) of *p*-dihydrocitronellyloxyphenol (No. 25) differs considerably from the value for the isomeric *p*-*n*-decyloxyphenol, which should be + 0.038 (readily calculated from the data on alkoxyphenols in Table I). Similarly, the R_M values for *p*-hexahydrofarnesyloxyphenol (No. 26) and *p*-dihydrophytyloxyphenol (No. 27) are + 0.431 and + 0.947 respectively, whereas the calculated value for their corresponding straight-chain isomers would be + 0.682 for *p*-*n*-pentadecyloxyphenol and + 1.328 for *p*-*n*-eicosyloxyphenol. Analysis of all the results in this section of Table I shows that if there are n branchings in a chain, there are n — 1 effects on R_M. In the case of System 2, therefore, there is a new parameter to be considered, ΔR_M(branching). Its mean value can be readily obtained from the data already discussed and is found to be — 0.130. The nature of the branching effect and the reason why it had not been observed in System 1 with the lower-molecular weight compounds proved puzzling for a time. It was considered as a possibility, for example, that the branching effect might only occur in systems in which the mobile phase was relatively non-aqueous; *i.e.* that the emergence of the new parameter was actually caused by the system change. To study this possibility, several lower-molecular weight compounds were converted to benzoates and run together with the higher-molecular weight compounds in System 2. It is clear, however, from comparison of the R_M values of *p*-*n*-butylphenyl benzoate (No. 35) and *p*-*tert.*-butylphenyl benzoate (No. 34) that the small difference between them can only be attributed to the slight effect of the ΔR_M(H) parameter and is not nearly large enough to be due to the branching effect. Other work, not shown here, confirmed that the branching effect was in fact not produced by any particular chromatographic system, although its magnitude (in common with other ΔR_M values) was influenced by the nature of the system. The next possibility to be investigated was that, for some reason, the branching effect only manifested itself in isoprenoid type chains containing at least ten carbon atoms. In order to examine this point, we synthesized *p*-(3,5,5-trimethylhexyloxy)-phenol (No. 22), which contains a 9-carbon chain and two chain branchings. This compound has an R_M value of — 0.225. The R_M value of the unbranched *p*-*n*-nonyloxyphenol, on the other hand, would be — 0.086 (this compound was unavailable, but its R_M value can be easily and accurately calculated from the data on the homologous members of this series). The theoretical R_M value of compound No. 22, subtracting *one* increment for ΔR_M(branching), would be — 0.086 — 0.130 = — 0.216, almost identical with the actual experimental R_M value for this compound. (It is important to

note that although compound No. 22 contains a quaternary carbon atom, from the chromatographic point of view this must only be counted as one branch.) This calculation demonstrates clearly that the branching effect is not a characteristic only of isoprenoid chains. Further confirmation of this point was obtained by comparing the R_M values of ergosterol and 7-dehydrocholesterol (Nos. 53 and 54). Although these substances are quite different from the others in our series, they were used because they are convenient sources of a 9-carbon chain and an 8-carbon chain. Ergosterol was an especially valuable compound to correlate, since it contains two *vicinal* branches. Calculation of the R_M value of ergosterol from 7-dehydrocholesterol is given in Table II.

TABLE II

CALCULATION OF R_M FOR ERGOSTEROL

Constituents	*Increment*	
	+	−
R_M(7-dehydrocholesterol)	1.016	
+ $\Delta R_M(CH_2)$	0.129	
+ ΔR_M(double bond)		0.080
+ ΔR_M(branching)		0.130
Sum of increments	1.145	0.210
Calculated R_M(ergosterol)	= +0.935	
Experimental R_M	= +0.947	

The agreement is excellent and shows that the non-isoprenoid side-chain in ergosterol, containing two vicinal branches, also exhibits only one branching effect on the R_M value, confirming that the "n — 1 effect" is independent of the relative positions of multiple branchings.

From these experiments, therefore, the nature of the branching effect on chromatography in reversed phase systems can be stated as follows. When a compound contains an alkyl chain with at least two branches, its R_M value is decreased by an increment that is a constant for the system. If there are n branchings, there are n — 1 increments that reduce the R_M value. The value of this parameter is unaffected by the relative positions of the branchings, their structure, or by the length of the alkyl chain. In System 2, ΔR_M(branching) is equal, but opposite in sign to, $\Delta R_M(CH_2)$; this relationship, however, can be expected to be different in other types of chromatographic system (see the results in System 6). In direct phase systems, branching (after the first) must increase R_M, providing the system is suitable for observing the effect.

The effect of branching on R_M can be related to the fact that, in aliphatic hydrocarbons, branched members have a smaller molar volume than unbranched members. The molar volume of a compound is normally determined in the gas state and is affected by all branchings. In chromatography, however, where substances are studied in the liquid state, entropy effects may play a greater part. It is perhaps due to such a consideration that only n — 1 branchings are effective in chromatography. Thus, the first branch in any chain can always be considered as terminal and subject to free

rotation. A second branch in the chain must introduce a hindrance to rotation, with a resultant effect on entropy.

If the branching forms part of a ring system, as in *p*-cyclohexylhexyloxyphenol (No. 21), the same rule applies: the ring counts as one branch only. Hence there is no ΔR_M increment in this compound, and its R_M value (+ 0.228) is almost exactly as calculated by subtracting $2 \times \Delta R_M$(H) from the R_M value of the straight-chain compound, *p*-*n*-dodecyloxyphenol (No. 11).

Calculation of R_M values for System 2

We have not calculated the R_M values of all the fifty-four compounds listed in Table I, as many of them have been used to provide the data for the calculation of the various ΔR_M parameters. Two fairly complex compounds were, however, chromatographed in System 2 in order to test the method of structural analysis in this system and particularly the use of the new ΔR_M(branching) parameter. These were tocol, the parent member of the vitamin E series, and the important naturally-occurring substance, vitamin A alcohol. The calculations for these substances are given below.

(*i*) *Tocol.* This substance (No. 50) is 2-methyl-2-(4',8',12'-trimethyltridecyl)-6-chromanol. Its empirical formula is $C_{26}H_{41}O_2$ and it can be considered as a complex derivative of phenol. Its R_M value can be found from that of phenol, and the R_M values for the other tocopherols can all in turn be calculated from that of tocol (Table III).

(*ii*) *Vitamin A.* The R_M value of vitamin A could be calculated from R_M(ethanol)

TABLE III

CALCULATION OF R_M FOR TOCOL

Constituents	Increment +	Increment −
R_M(phenol)*		1.125
+ ΔR_M(O in OCR_3)		0.329
+ ΔR_M(20 carbon atoms + 39 hydrogen atoms) $\simeq$ 20 × $\Delta R_M(CH_2) - \Delta R_M$(H)**	2.560	
+ 2 × ΔR_M(branching)		0.260
Sum of increments	2.560	1.714
Calculated R_M(tocol)	= +0.846	
Experimental R_M	= +0.806	

* Phenol itself was not chromatographed in System 2, as its R_F value is rather too high and in fact is likely to be less accurate when found experimentally than can be calculated by extrapolation from the data on the higher phenols (compounds Nos. 1–4). For this reason we have used the latter data to provide R_M(phenol) by simple extrapolation.

** For the validity of this approximation in System 2 see text.

if the necessary data were available. In this study, however, we have not studied a sufficient number of alcohols to determine the value of ΔR_M(primary OH), so have calculated from the R_M value of phytol (No. 48), a long-chain poly-isoprenoid alcohol. The calculation is given in Table IV.

TABLE IV

CALCULATION OF R_M FOR VITAMIN A

Constituents	*Increment +	*Increment −
R_M(phytol)	0.218	
$-10 \times \Delta R_M$(H)		0.400
$-\Delta R_M$(branching) *	0.130	
Sum of increments	0.348	0.400
Calculated R_M(vitamin A)	= −0.052	
Experimental R_M	= −0.040	

* The phytol molecule contains 4 chain branches. Vitamin A contains 2 chain branches and one ring, which (see text) also counts as one branch. The difference between them therefore corresponds to one effective branching unit: since vitamin A has less branches than phytol, the ΔR_M parameter, which is negative, is added.

TABLE V

CHROMATOGRAPHY OF SOME HYDROQUINONE MONO-ETHERS AND ALKYL *p*-NITROBENZOATES IN SYSTEM 3

Stationary phase: Whatman No. 4 paper impregnated with a 5% (v/v) solution of olive oil in light petroleum.
Mobile phase: 50% (v/v) ethanol in water.

No.	Compound	R_F	R_M
	Ethers		
5	*p*-Butoxyphenol	0.75	−0.469
6	*p*-(3-Methylbutoxy)-phenol	0.63	−0.224
7	*p*-Hexyloxyphenol	0.49	+0.021
8	*p*-Heptyloxyphenol	0.35	+0.267
9	*p*-Octyloxyphenol	0.235	+0.512
13	*p*-(Pent-4-enyloxy)-phenol	0.70	−0.375
14	*p*-(Hex-4-enyloxy)-phenol	0.58	−0.134
19	*p*-Cyclopentyloxyphenol	0.80	−0.600
20	*p*-Cyclohexyloxyphenol	0.70	−0.380
22	*p*-(3,5,5-Trimethylhexyloxy)-phenol	0.24	+0.498
23	*p*-Geranyloxyphenol	0.26	+0.447
24	*p*-Citronellyloxyphenol	0.20	+0.602
25	*p*-Dihydrocitronellyloxyphenol	0.15	+0.747
55	*p*-(1-Methylbutoxy)-phenol	0.75	−0.469
56	*p*-(2-Methylbutoxy)-phenol	0.63	−0.224
57	*p*-(1-Ethylbutoxy)-phenol	0.63	−0.224
58	*p*-(1-Methylpentyloxy)-phenol	0.63	−0.224
59	*p*-(1-Ethylpentyloxy)-phenol	0.49	+0.021
60	*p*-(1-Propylbutoxy)-phenol	0.49	+0.021
61	*p*-Sorbyloxyphenol	0.63	−0.227
	Esters		
62	Ethyl *p*-nitrobenzoate	0.37	+0.238
63	Propyl *p*-nitrobenzoate	0.24	+0.497
64	Allyl *p*-nitrobenzoate	0.295	+0.380
65	Propargyl *p*-nitrobenzoate	0.36	+0.246

RESULTS WITH SYSTEM 3

System 3 was 50% (v/v) aqueous ethanol against olive oil. Several compounds that had already been chromatographed in System 2 were run in this system, in order to obtain additional information about the effect of changes in the ethanol concentration of the mobile phase on the ΔR_M parameters. System 3 was also used to study one or two other aspects of the unsaturation and branching effects. The results are given in Table V.

The $\Delta R_M(CH_2)$ parameter

In System 3, $\Delta R_M(CH_2)$, calculated from compounds 5–9, was found to be 0.245 ± 0.001.

Unsaturation

The principle of independent contributions of carbon and hydrogen atoms to R_M suggests that the ΔR_M increment for a triple bond should be calculable in the same way as for a double bond, that is, $\Delta R_M(C{\equiv}C)$ should be equivalent to $\Delta R_M(C{=}C) - 2 \times \Delta R_M(H)$. Propargyl alcohol was a convenient acetylenic compound, but we were unable to prepare propargyloxyphenol for comparison with the other hydroquinone mono-ethers. Propargyl alcohol was therefore studied as its *p*-nitrobenzoate (No. 65) and compared with the *p*-nitrobenzoates of ethanol, propanol, and allyl alcohol. $\Delta R_M(C{=}C)$ was calculated by comparing, in the usual way, the R_M values of pairs of compounds, differing only in the presence of one double bond. Thus from comparison of compounds No. 7 and 14, 23 and 24, 24 and 25, $\Delta R_M(C{=}C)$ is -0.152 ± 0.007. The experimental R_M value for propargyl *p*-nitrobenzoate differs from that of the allyl ester by -0.134, almost exactly that required for the further increment due to loss of two hydrogen atoms. This confirms that the acetylenic function can be calculated in the same way as the olefinic function by the method of atomic ΔR_M parameters. It should be noted, however, that the difference in R_M values between the allyl and propyl esters is only -0.097. It was found previously[1] in System 1 that allyl-substituted phenols ran slightly faster than required by theory and it was suggested that the effect was due to resonance in the allyl group. From the admittedly rather slender evidence of compound 64, it would seem that a similar effect might exist even in the allyl ester; here, although the allyl group is separated from the aromatic ring it is possible for conjugation of the allyl group with the ring to take place through the lone pair of electrons on the oxygen atom of the ester grouping. It follows, moreover, from the fact that the allyl and propargyl compounds can be correlated, that propargyl compounds can also be expected to show the "allyl" effect and run slightly faster than required by theory.

Another question was whether $\Delta R_M(C{=}C)$ remained constant if two or more bonds were conjugated with one another. In order to examine this, *p*-sorbyloxyphenol, which contains two conjugated double bonds, was prepared. Its R_M value was -0.227, and the theoretical R_M value for this compound (derived by calculation from *p*-hexyloxyphenol and *p*-(hex-4-enyloxy)-phenol) is -0.286. Considering the dimension of $\Delta R_M(CH_2)$ in System 3, this cannot be taken as seriously in error. There is evidently no major effect of conjugation on $R_M(C{=}C)$—see also the discussion[1] on propenylphenol in System 1—and this is confirmed by the calculation for vitamin A, which contains five conjugated double bonds.

Branching in ethers

Six new ethers (compounds Nos. 55–60) were prepared and chromatographed in System 3, in order to examine whether the *size* of the branched chain in secondary ethers affected the value of ΔR_M(O). As seen from Table V, the only primary ether in this group, *p*-(2-methylbutoxy)-phenol (No. 56) runs slower than the isomeric secondary ether (No. 55). The five secondary ethers show a constant homologous $\Delta R_M(CH_2)$ increment of + 0.245, irrespective of the nature of the secondary branching at oxygen. Thus the two isomeric secondary hexyl ethers, compounds 57 and 58, have identical R_M values and so do the two secondary heptyl ethers, compounds 59 and 60.

RESULTS WITH SYSTEM 4

In Table VI the results on 11 compounds run in System 4 (90 % ethanol against olive oil) are given. The system was studied to provide yet another bridge between the low molecular weight phenols and the more complex molecules studied subsequently,

TABLE VI

CHROMATOGRAPHY OF HYDROQUINONE MONO-ETHERS AND TOCOPHEROLS IN SYSTEM 4

Stationary phase: Whatman No. 4 paper impregnated with a 5% v/v solution of olive oil in light petroleum.
Mobile phase: 90% v/v ethanol in water.

No.	*Compound*	R_F	R_M
11	*p*-Dodecyloxyphenol	0.70	—0.357
12	*p*-Tetradecyloxyphenol	0.59	—0.155
15	*p*-Hexadecyloxyphenol	0.48	+0.041
16	*p*-Octadecyloxyphenol	0.36	+0.258
26	*p*-Hexahydrofarnesyloxyphenol	0.64	—0.250
27	*p*-Dihydrophytyloxyphenol	0.44	+0.107
50	Tocol	0.50	0.000
51	δ-Tocopherol (8-methyltocol)	0.45	+0.091
66	β-Tocopherol (5,8-dimethyltocol)	0.37	+0.228
52	γ-Tocopherol (7,8-dimethyltocol)	0.37	+0.228
67	α-Tocopherol (5,7,8-trimethyltocol)	0.31	+0.342

it being necessary to ensure that the additivity principle could be applied over the whole range of polarity of the mobile phase. The results again confirm that MARTIN's equation is obeyed: $\Delta R_M(CH_2)$ was constant to well within experimental error right up to *p*-octadecyloxyphenol, and was equal to + 0.103 ± 0.006. Note, however, the branching effects in compounds 26 and 27, as before. Table VII summarizes the data on some important parameters for Systems 2, 3 and 4.

RESULTS WITH SYSTEM 5

In this system, the stationary phase was changed to the non-polar liquid paraffin, which is normally used for the chromatography of the tocopherols, ubiquinones and

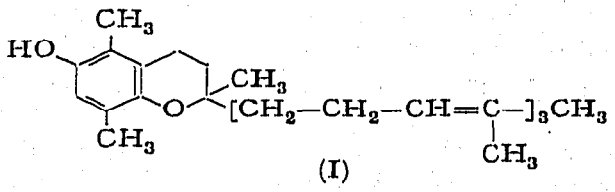
TABLE VII

VARIATION OF ΔR_M PARAMETERS OF SOME GROUPS AND STRUCTURAL FEATURES WITH CHANGE OF ETHANOL CONCENTRATION OF MOBILE PHASE IN SYSTEMS 2, 3 AND 4

Structural unit	ΔR_M *in system*		
	2	*3*	*4*
CH_2	+0.129	+0.245	+0.103
Ring-attached CH_2	+0.130		+0.097
Double bond (—2H)	—0.080	—0.152	
Branching (n—1)	—0.130	—0.255	—0.104

ubichromenols. In the first study, 65 % ethanol was used as mobile phase. Table VIII gives the data on 10 compounds. The relative positions of the substances remain the same as in previous systems, but note the restricted range of the chromatograms, leading to a value for $\Delta R_M(CH_2)$ that is now as large as it was in System 1. Note also the large difference (0.411) between the R_M values of tocol and *p*-dihydrophytyloxyphenol in this system. Although the two substances only differ by 2 hydrogen atoms in their empirical formula (compare the R_M values of compounds 70 and 71, for example, which differ by only 0.204), tocol is a tertiary ether (chromanol) whereas *p*-dihydrophytyloxyphenol is a primary ether.

The data in Table VIII were used for the elucidation of the structure of ε-tocopherol. We have shown elsewhere[8] that natural ε-tocopherol is not a homologue of tocol, as had previously been thought, but in fact has the structure (I).



(I)

This structure can be assigned to ε-tocopherol on chromatographic evidence[9]. Since ε-tocopherol can be hydrogenated to a substance having the same R_M value as β-tocopherol (it is in fact identical with β-tocopherol, as shown by other evidence), the ΔR_M change can be regarded as due to the presence of unsaturation in the former molecule. This value, ΔR_M(β-tocopherol—ε-tocopherol) = + 0.596, is almost exactly the required shift in R_M for three double bonds, which is + 0.612.

RESULTS WITH SYSTEM 6

Study of the high molecular weight ubiquinones, vitamins K and the ubichromenols requires liquid paraffin as stationary phase and 95 % ethanol as mobile phase. The results in this system are given in Table IX. The following sections describe in detail the methods of structural analysis used and show how the R_M values of these complex molecules can be calculated.

TABLE VIII

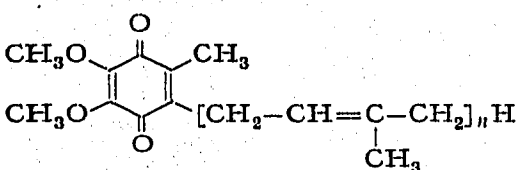
CHROMATOGRAPHY OF HYDROQUINONE MONO-ETHERS AND TOCOPHEROLS IN SYSTEM 5

Stationary phase: Whatman No. 4 paper impregnated with a 5 % v/v solution of liquid paraffin in light petroleum.
Mobile phase: 65 % (v/v) ethanol in water.

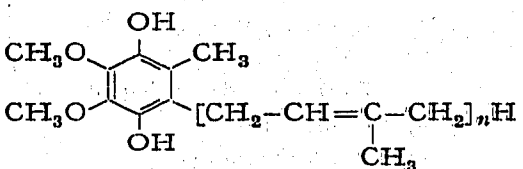
No.	*Compound*	R_F	R_M
50	Tocol	0.65	—0.268
51	δ-Tocopherol	0.425	+0.130
66	β-Tocopherol	0.22	+0.550
52	γ-Tocopherol	0.22	+0.550
67	α-Tocopherol	0.10	+0.956
68	Natural ε-tocopherol	0.53	—0.046
69	Hydrogenated ε-tocopherol	0.22	+0.550
27	*p*-Dihydrophytyloxyphenol	0.42	+0.143
70	4-Methoxy-2-methyl-5-phytylphenol	0.285	+0.398
71	4-Methoxy-2-methyl-5-dihydrophytyl-phenol	0.20	+0.602

Structures of the compounds listed in Table IX

In order to make the structural analyses and calculations more clear, we have depicted below the structures of some of the key compounds of Table IX, with some details of their interrelationships. Ubiquinones 30, 45 and 50 have structure (II) (n = 6, 9 and 10, respectively).

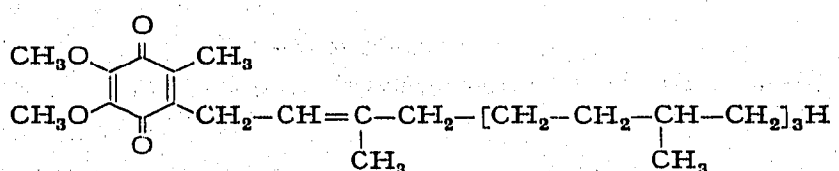


(II)



(III)

The analogous ubiquinols 30, 45 and 50 have structure (III). Dodecahydroubiquinone 30 and dodecahydroquinol 30 are derived from (II) and (III) respectively by reduction of the side-chains. Hexahydroubiquinone 20 (IV) is an allyl-type substituted quinone and octahydroubiquinone 20 is the analogous compound with a saturated side-chain.



(IV)

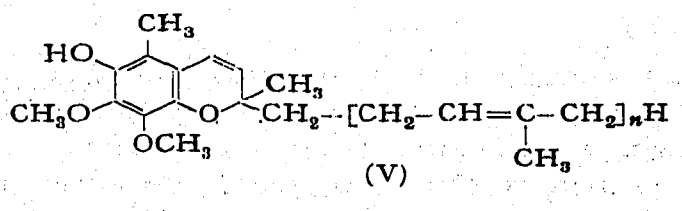
Ubichromenols 20, 30 and 50 have structure (V) (n = 3, 5 and 9, respectively).

TABLE IX

CHROMATOGRAPHY OF UBIQUINONES, UBICHROMENOLS, TOCOPHEROLS, VITAMINS K, PHENYL PALMITATES AND POLYNUCLEAR HYDROCARBONS IN SYSTEM 6

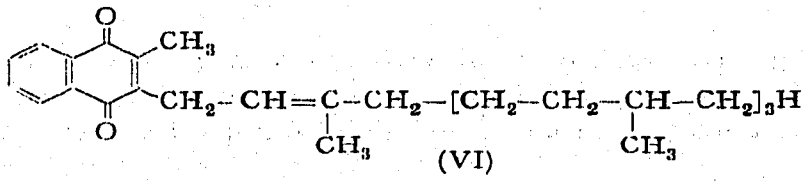
Stationary phase: Whatman No. 4 paper impregnated with a 5% (v/v) solution of liquid paraffin in light petroleum.
Mobile phase: 95% (v/v) ethanol in water.

No.	Compound	R_F	R_M
	Tocopherols and their ethers		
67	α-Tocopherol	0.85	—0.746
72	β-Tocopherol allyl ether	0.26	+0.452
73	γ-Tocopherol allyl ether	0.33	+0.301
74	Tocol allyl ether	0.40	+0.167
	Palmitates		
75	Phenyl palmitate	0.58	—0.138
76	*p*-Cresyl palmitate	0.50	0.000
	Hydrocarbons		
77	Anthracene	0.85	—0.770
78	Phenanthrene	0.85	—0.770
79	Benzanthracene	0.75	—0.481
80	Pyrene	0.75	—0.481
	Quinones and quinols		
81	Vitamin K_1	0.425	+0.127
82	Vitamin K_2	0.22	+0.566
83	2-Methyl-5-dihydrophytylbenzoquinone	0.51	—0.046
84	Hexahydroubiquinone 20	0.73	—0.434
85	Octahydroubiquinone 20	0.67	—0.314
86	Ubiquinone 30	0.65	—0.276
87	Dodecahydroubiquinone 30	0.28	+0.418
88	Dodecahydroubiquinol 30	0.72	—0.398
89	Ubiquinol 30	0.92	—1.046
90	Ubiquinone 45	0.25	+0.477
91	Ubiquinone 50	0.16	+0.720
92	Ubiquinol 50	0.555	—0.097
	Chromenols and chromanols		
93	Hexahydroubichromenol 20	0.85	—0.740
94	Hexahydroubichromanol 20	0.81	—0.627
95	Ubichromenol 30	0.79	—0.569
96	Ubichromenol 50	0.27	+0.428
	Hydroquinone mono-ether		
71	4-Methoxy-2-methyl-5-dihydrophytylphenol	0.91	—1.010

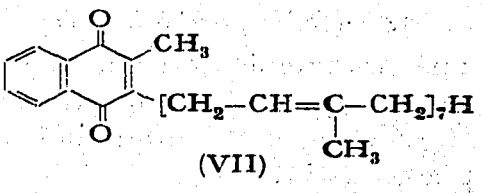


(V)

Although ubichromenol 20 itself was not available, hexahydroubichromenol 20 can be readily prepared from the available quinone and has structure (V) ($n = 3$) with a saturated side-chain. Hexahydroubichromanol 20 is the chromanol derived by reduction of the ring double bond. Vitamins K_1 and K_2 have structures (VI) and (VII), respectively.



(VI)



(VII)

Methods of structural analysis for compounds in System 6

(a) The "isoprene" unit

The tocopherols, ubiquinones, ubichromenols and vitamins K are all partly isoprenoid in structure and contain branched alkyl chains built up from saturated or unsaturated "isoprene" units. Thus α-tocopherol contains three saturated units, vitamin K_1 three saturated and one unsaturated unit, and ubiquinone 50 ten unsaturated units. For R_M calculations, it was convenient, therefore, to determine two new group parameters, ΔR_M ("isoprene" unit) and ΔR_M (hydrogenated "isoprene" unit). This eliminates the accumulation of small errors introduced when adding large numbers of $\Delta R_M(CH_2)$ and ΔR_M (CH) values and increments for double bonds, and branching effects. (Note that no "branching effect" error is introduced by this procedure as, in each case, the fusion of the isoprenoid chain with the ring constitutes the first, ineffective branch.) The new parameters were found from two series of compounds that, chromatographically, differ considerably, the ubiquinones and ubiquinols. The values from both series agreed well with each other. They are given in Table X.

TABLE X

ΔR_M PARAMETERS OF VARIOUS STRUCTURAL UNITS IN SYSTEM 6

Structural unit	ΔR_M
"Isoprene" unit in ubiquinones	+0.249
"Isoprene" unit in ubiquinols	+0.238
Hydrogenated "isoprene" unit	+0.366
Ring-attached CH_2 (tocopheryl ethers)	+0.142
Ring-attached CH_2 (aryl palmitates)	+0.138
CH=CH—CH=CH fused to an aromatic ring	+0.289
Double bond	−0.121
Branching effect*	−0.334
OCH_3 group vicinal to C=O in ubiquinones	−0.134

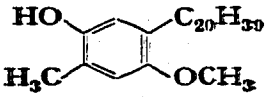
* It was assumed that ΔR_M (ring-attached CH_2) = ΔR_M (CH_2) = + 0.140 (see text).

(b) ΔR_M(ring-attached CH_2)

Many of the compounds contain nuclear-substituted methyl groups, and the parameter, ΔR_M(ring-attached CH_2), must be found for System 6. The tocopherols themselves, which differ in ring methyl groups and can therefore provide this parameter, run rather fast in this system, so three tocopheryl ethers were used. Because of our previous demonstrations that $\Delta R_M(CH_2)$ is strictly additive, we were confident that the value obtained from the ethers would be identical with that in the hydroxy compounds. To provide an additional check, however, the parameter was calculated independently from a comparison of the R_M values of phenyl and *p*-cresyl palmitates, which were synthesised for this purpose. The agreement between the two series was excellent, as shown in Table X.

(c) ΔR_M(OCH_3 ortho to OH)

The ubiquinols contain methoxyl groups *ortho* to their two hydroxy groups. The calculation of this important parameter is described below: it was obtained from the R_M values of the ubiquinol series and the key ether, 4-methoxy-2-methyl-5-phytylphenol (VIII).



(VIII)

(d) ΔR_M (double bond)

This was found by comparing the R_M values of the ubiquinols with those of their perhydro compounds.

(e) ΔR_M(branching)

This parameter was calculated by comparing the R_M values of ubiquinones and the phenyl palmitates, as shown below. (Since no independent determination of $\Delta R_M(CH_2)$ was made in System 6, we have assumed that it has the same value as ΔR_M(ring-attached CH_2). This is certainly valid for this system, in which differences in the values for various ΔR_M(H) parameters must be insignificant.)

The mean value for $\Delta R_M(CH_2)$ from Table X is + 0.140. Hence,

ΔR_M for $CH_3—CH_2—CH_2—CH_2—CH_2$ $= 5 \times 0.140 = 0.700$

Experimental ΔR_M(saturated "isoprene" unit) $= +0.366$

Therefore,

ΔR_M(branching) $= +0.334$

(In this system the ratio of ΔR_M(branching) to $\Delta R_M(CH_2)$ is nearly twice as large as it was in the olive oil systems—see Table VII.)

(f) ΔR_M(OCH_3 ortho to C=O)

This parameter was obtained from the R_M data on perhydroubiquinone 20 and 2-methyl-5-dihydrophytylbenzoquinone.

$$R_M(\text{perhydroubiquinone 20}) = R_M(\text{2-methyl-5-dihydrophytylbenzoquinone}) + 2 \times \Delta R_M(OCH_3 \textit{ ortho} \text{ to } C{=}O)$$

Therefore,

$$\Delta R_M(OCH_3 \textit{ ortho} \text{ to } C{=}O) = \frac{-0.314 + 0.046}{2} = -0.134$$

(g) $\Delta R_M(CH{=}CH{-}CH{=}CH)$

This is a new group ΔR_M parameter and is of value in the calculation of vitamins K. The latter are all alkylated naphthaquinones and can be correlated with the ubiquinones through the formal fusion of a new aromatic ring to the existing quinonoid structure in the latter. (Note that, as discussed previously[1], if polarizations in molecules are not pronounced, R_M values can be calculated from these formal structural differences, and they are not influenced by the chemical or electronic changes involved in "aromaticity".)

It is possible, without introducing any serious error, to calculate the new parameter independently from $\Delta R_M(CH_2)$ itself.
Thus

$$\Delta R_M(CH{=}CH{-}CH{=}CH) \simeq 4 \times \Delta R_M(CH_2) + 2 \times \Delta R_M(\text{double bond}) \simeq +0.560 - 0.242 = +0.318$$

However, when dealing with a new ΔR_M parameter, it is preferable, if possible, to check it unequivocally, since an unforeseen interaction can never be ruled out. To do this, we chromatographed a series of polynuclear aromatic hydrocarbons in System 6 and calculated as follows.

$$\Delta R_M(CH{=}CH{-}CH{=}CH) = R_M(\text{benzanthracene}) - R_M(\text{anthracene}) = +0.289$$

It will be seen that the value is indeed very close to the approximation calculated directly from $\Delta R_M(CH_2)$ above. (Note that anthracene and phenanthrene on the one hand and benzanthracene and pyrene on the other are chromatographically indistinguishable, confirming our views on the irrelevance of pure energy characteristics (in the absence of other effects) on chromatographic behaviour.)

Calculations of R_M values for complex molecules in System 6

Calculations of R_M(vitamin K_2) from R_M(ubiquinone 50) and from R_M(vitamin K_1)

These calculations are given in Tables XI and XII. The excellent agreement between these two calculations provides further evidence of the precise convertibility of R_M data from series to series.

Calculation of R_M(ubichromenol 50) from R_M(α-tocopherol) and hence from R_M(phenol)

This is the most extensive calculation we have attempted. It demonstrates the importance of evaluating every new constitutive effect in a molecule. Ubichromenol 50 has a molecular weight of 862.

1st calculation. To calculate the R_M value of ubichromenol 50 from that of α-tocopherol, the effect of the following molecular modifications, in terms of ΔR_M parameters, must be known.

1. Subtracting two CH_3 groups from the ring.
2. Adding two OCH_3 groups to the ring, one *ortho* to the hydroxy group.
3. Adding one double bond to convert from a chromanol to a chromenol.

TABLE XI

CALCULATION OF R_M(VITAMIN K_2) FROM R_M(UBIQUINONE 50)

Constituents	*Increment* +	*Increment* —
R_M(ubiquinone 50)	0.720	
$-2 \times \Delta R_M(OCH_3)$	0.268	
$+ \Delta R_M$(fused ring)	0.289	
$-3 \times \Delta R_M$("isoprene" unit)		0.747
Sum of increments	1.277	0.747
Calculated R_M(vitamin K_2)		$= +0.530$
Experimental R_M		$= +0.556$

TABLE XII

CALCULATION OF R_M(VITAMIN K_2) FROM R_M(VITAMIN K_1)

Constituents	*Increment* +	*Increment* —
R_M(vitamin K_1)	0.127	
$+3 \times \Delta R_M$("isoprene" unit)	0.747	
$+3 \times \Delta R_M$(double bond)		0.363
Sum of increments	0.874	0.363
Calculated R_M(vitamin K_2)		$= +0.511$
Experimental R_M		$= +0.556$

TABLE XIII

FIRST CALCULATION OF R_M(UBICHROMENOL 50) FROM R_M(α-TOCOPHEROL)

Constituents	*Increment* +	*Increment* —
R_M(α-tocopherol)		0.746
$-2 \times \Delta R_M$(ring-attached CH_2)		0.280
$+2 \times \Delta R_M(OCH_3)$		0.268
$+6 \times R_M$("isoprene" unit)	1.494	
$+4 \times R_M$(double bond)		0.484
Sum of increments	1.494	1.778
Calculated R_M(ubichromenol 50)	$= -0.284$	
Experimental R_M	$= +0.428$	

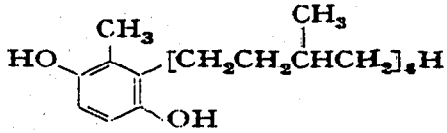
4. Adding a further three double bonds to convert the saturated side-chain to a tri-isoprenoid unsaturated side-chain.

5. Adding a further 6 unsaturated "isoprene" units to increase the chain length.

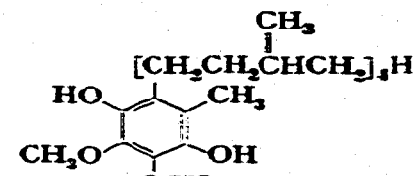
The only ΔR_M parameter whose precise value remained in doubt was ΔR_M(OCH$_3$ *ortho* to OH). Because of hydrogen-bonding and the possibility of a pronounced *ortho*-effect it could be expected to be of great importance in the calculation. Before compound 71 was available, this parameter could not be calculated and it was first thought it might be satisfactory to use a value for a similar grouping, *i.e.* the known parameter, given in Table X, for ΔR_M(OCH$_3$ vicinal to >C=O in quinones). The calculation is given in Table XIII.

It is clear that there is a serious discrepancy between the calculated and experimental R_M values. The error must arise because the interaction between the OCH$_3$ group and the OH group is, as expected, considerably different from that between the OCH$_3$ group and the C=O group. The *ortho*-effect between the latter two groups must be large in this system.

2nd calculation. The calculation shown above is in error by an amount equivalent to about four CH$_2$ groups and it is clear that ΔR_M(OCH$_3$ *ortho* to OH) must be determined with much greater accuracy. This could be done, by the normal procedure of formal structural analysis, by comparing the R_M values of two suitably substituted phenols, one of which must contain suitably orientated OCH$_3$ groups. Amongst the range of compounds considered as possibly being available were 2-dihydrophytyl-3-methylhydroquinone (IX) and 2-dihydrophytyl-5,6-dimethoxy-3-methylhydroquinone (X).



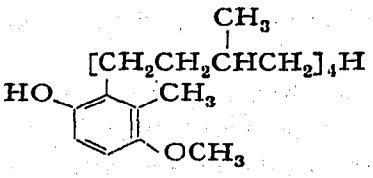
(IX)



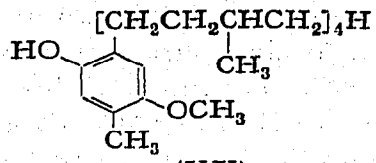
(X)

The orientation of these two compounds is very similar to that in tocopherol and ubichromenol respectively. The difference in R_M between the two compounds would be due only to the two OCH$_3$ groups, and ΔR_M(IX—X) would be equal to twice ΔR_M(OCH$_3$ *ortho* to OH). There were two practical difficulties, however. First, even if the two compounds could be prepared, they would be unlikely to chromatograph in System 6, since they each contain two OH groups. This could be overcome by preparing the 1-methyl ether of (IX) and (X) respectively and the resulting ethers would have the further advantage of resembling tocopherol and ubichromenol (both of which are cyclic mono-ethers) even more closely. Secondly, however, although (X) was available through the reduction of the corresponding octahydroubiquinone 20 (No. 85), (IX) could not be readily synthesised since entry of the phytyl group in the 3-position is sterically hindered. The problem was solved in the following manner.

(*i*) *Hypothetical R_M values for (IX) and (X) in System 6.* Although the required ether of (IX), 2-dihydrophytyl-4-methoxy-3-methylphenol (XI), is difficult to synthesise, its isomer, 6-dihydrophytyl-4-methoxy-3-methylphenol (XII) was readily obtained by condensation of toluquinol 1-methyl ether and phytol, followed by hydrogenation of the phytyl group.



(XI)



(XII)

This compound (No. 71) was prepared and had an R_M value of — 1.010 in System 6. Previous work[1] had already shown that differences in the orientation of alkyl groups do not affect R_M values of alkoxyphenols. Therefore it could be safely assumed that, if it were available (XI) would also have an R_M value of — 1.010 in System 6. If the ΔR_M increment were now known for the change involved in converting an OH group to an OCH_3 group, it would be possible to calculate the *hypothetical* R_M value for compound (IX) from the R_M value of (XII). This increment was obtained by comparing the R_M values of α-tocopherol and β-tocopheryl allyl ether, as follows:

(ii) Calculation of R_M(β-tocopherol) from R_M(α-tocopherol)

$$R_M(\beta\text{-tocopherol}) = R_M(\alpha\text{-tocopherol}) - \Delta R_M(\text{ring-attached } CH_3)$$
$$= -0.746 - 0.140 = -0.886$$

(iii) Calculation of R_M(β-tocopheryl methyl ether) from R_M(β-tocopheryl allyl ether)

$$R_M(\text{methyl ether}) = R_M(\text{allyl ether}) - 2 \times R_M(CH_2) - R_M(\text{double bond})$$
$$= +0.452 - 0.280 + 0.121 = +0.293$$

Therefore,

$$\Delta R_M(\text{effect of methylating OH group}) = R_M(\beta\text{-tocopheryl methyl ether}) - R_M(\beta\text{-tocopherol})$$
$$= +0.293 + 0.866 = +1.179$$

(Note: if the methyl ether of β-tocopherol had been available, the difference could have been found directly by chromatographing it with β-tocopherol. This calculation illustrates the interconvertibility of R_M data among related series of compounds.)

(iv) Calculation of R_M values for (IX) and (X)

$$R_M(\text{IX}) = R_M(\text{XI}) - 1.179$$
$$= -2.189$$

which would be the R_M value of (IX) if it could be run in System 6, and R_M(X) can now be calculated from R_M(dodecahydroubiquinol 30), (No. 88), by the usual method, as follows:

$$R_M(\text{X}) = R_M(\text{dodecahydroubiquinol 30}) - 2 \times \Delta R_M(\text{hydrogenated "isoprene" unit})$$
$$= -0.398 - 0.732 = -1.130$$

which would be the R_M value of (X) if it could be run in System 6.

(v) Calculation of ΔR_M(OCH_3 ortho to OH)

$$\Delta R_M(OCH_3 \textit{ ortho to } OH) = \frac{R_M(\text{X}) - R_M(\text{IX})}{2} = +0.530$$

(*vi*) *Calculation of R_M(ubichromenol 50)*. The calculation is as previously, using the new parameter for OCH_3 (Table XIV).

The agreement is good, considering the lengthy procedure involved. By similar methods it is possible to correlate the R_M values of all the tocopherols, tocotrienols, vitamins K, ubiquinones, ubichromenols and members of related series of compounds. For example, R_M(ubichromenol 20) can now be calculated from R_M(ubichromenol 50) by subtracting the R_M increment for six unsaturated units (1.494). The calculated value is found to be — 0.751, in excellent agreement with the experimental R_M value of this compound, which is — 0.740. It is clear that, with adequate chromatographic data and with a certain amount of information about the functional groups present, the R_M values of some of these complex molecules can be calculated to within a small fraction of a carbon atom.

TABLE XIV

SECOND CALCULATION OF R_M(UBICHROMENOL 50) FROM R_M(α-TOCOPHEROL)

Constituents	*Increment*	
	+	—
R_M(α-tocopherol)		0.746
— 2 × R_M(ring-attached CH_2)		0.280
+ 4 × ΔR_M(double bond)		0.484
+ ΔR_M(OCH_3)		0.134
+ ΔR_M(OCH_3 *ortho* to OH)	0.530	
+ 6 × ΔR_M("isoprene" unit)	1.494	
Sum of increments	2.024	1.644
Calculated R_M(ubichromenol 50)	= +0.380	
Experimental R_M	= +0.428	

DISCUSSION

In principle it should now be possible to accept MARTIN's postulate as to the constancy of ΔR_M values in any molecule and in any system, providing that constitutive effects do not occur. If these do occur, they can often be adequately accounted for, as we have shown here and previously[1]. It is thus possible to calculate the R_M values of many complex molecules from data derived from relatively simple compounds, providing that chromatographic conditions are near-ideal and have been shown to yield accurate R_M values[1]. The recent work of HOWE[10] must be considered in this connection since this author, after his most extensive study on over 100 organic acids in several series, did not find agreement with MARTIN's equation. Two points, however, arise from HOWE's study. First, in some of his series, R_F values rapidly approached a limiting value after 8 carbon atoms. Since this value was about 0.80, this is strongly indicative of the non-ideal conditions that exist near the moving front of chromatograms due to excessive evaporation and other factors. As we have already suggested[1], R_F values of this order are likely to be subject to considerable error under tank con-

ditions and with certain systems, an R_F value of about 0.80 may appear to be the limiting R_F obtainable irrespective of the homologous increment. It is important, therefore, to stress that, providing the system is chosen so that R_F values fall within the workable range, there is apparently no limitation on MARTIN's postulate with respect to homologous addition. Thus HOWE was able to chromatograph dicarboxylic acids up to 10 carbon atoms in length and obtain a linear plot of R_M when the maximum R_F value was 0.53. As we have shown here, the homologous increment ΔR_M-(CH_2) is constant up to a chain length of 18 carbons (octadecyloxyphenol) and we have been able to calculate the R_M values of compounds containing branched side-chains of up to 50 carbon atoms. The second conclusion from HOWE's work was that $\Delta R_M(CH_2)$ varies from one homologous series to another. We regard this as primarily due to the nature of his series. As BARK AND GRAHAM[11] have shown, the paper chromatography of organic acids can be profoundly influenced by adsorption of the functional group on paper. In our own (unpublished) studies we have found that this is true *even in reversed phase systems*, where there is an inert stationary phase over the paper. It must be considered, therefore, that HOWE's results may have been affected in this manner and adsorption could account for the lack of constancy that he found for $\Delta R_M(CH_2)$. It is clear that where a possibility of adsorption exists, MARTIN's equation may not be precisely obeyed, even in homologous series.

The calculation of the R_M value for ubichromenol 50 illustrates that hypothetical R_M values can be calculated for compounds that could not be run in the system for which they have been calculated. These hypothetical R_M values can be dealt with arithmetically, as are real R_M values.

There are obvious advantages in being able to calculate the R_M values of complex molecules. We have already shown elsewhere[9] how such calculations can be used to determine unsaturation in molecules by purely chromatographic methods. They can also be used to obtain information about the structure of an unknown compound, even when it is available only in small amounts or is impure. It is often possible to eliminate alternative structures, such as might be proposed for a new or unknown compound of natural origin.

It may be possible, in the future, to choose a limited series of standard chromatographic system and determine, with accuracy, the values for all the important atomic group and constitutive ΔR_M parameters met with in simple series of compounds. Providing that the chromatographic systems and the conditions of running were both rigorously standardised, it might even be possible for this data to be used by different workers without the necessity of their frequent re-determination in individual laboratories. Reversed phase systems should be chosen as standard wherever possible and the mobile phase be restricted to one of two solvents, such as aqueous ethanol or acetone, which have exceptionally wide scope. With the exception of sugars and amino acids which, for structural analysis purposes as opposed to pure identification purposes, can in any case be handled as their derivatives, such reversed phase systems can deal with most classes of organic compound.

SUMMARY

Series of phenols, hydroquinone mono-ethers, esters, ethers, alcohols, tocopherols, quinones and chromenols were run in five chromatographic systems. Chromatographic

constancy was shown for ΔR_M increments due to the following groups and structural changes: H, CH_2, ring-attached CH_2, double bond, branching, oxygen in ethers, and the "isoprene" unit in long chains. MARTIN's equation was obeyed in all the systems studied. Methods of structural analysis are demonstrated by which the chromatographic behaviour of complex molecules can be accurately predicted from data derived from simple compounds and knowledge of the ΔR_M parameters.

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